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Plasma Tissue Factor Antigen Levels in Capillary Whole Blood and Venous Blood: Effect of Tissue Factor on Prothrombin Time

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To measure the amount of tissue factor released during specimen collection and its potential effect of shortening the prothrombin time, we measured tissue factor and prothrombin time in twenty-three paired venous and capillary blood samples from anticoagulated patients and in ten paired samples from controls. We also compared venous prothrombin time determined by a plasma-based assay with venous and capillary prothrombin time determined with a whole blood assay. Venous specimens were obtained using a two-syringe technique; capillary specimens were obtained by fingerstick after wiping the first drop of blood. Plasma tissue factor was determined by an enzyme-linked immunoabsorbant assay. The patients' mean venous tissue factor (235 ± 101 pg/ml) and capillary tissue factor (268 ± 106 pg/ml) were higher than those of the controls (161 ± 42 pg/ml and 187 ± 63 pg/ml, respectively, $P < 0.05$). These differences disappeared after adjusting for age. Capillary tissue factor levels were higher than venous tissue factor (244 ± 102 pg/ml vs. 213 ± 93 pg/ml), with a mean difference of 31 pg/ml ($P = 0.0001$). In addition, whole blood prothrombin time was lower in the capillary than in the venous samples (17.7 ± 5 sec vs. 18.3 ± 5.4 sec, $P = 0.004$). However, there was no correlation between capillary-venous differences in tissue factor and capillary-venous differences in the whole blood prothrombin time. Whole blood capillary and venous prothrombin times highly correlated with the plasma-based venous prothrombin time ($r = 0.98$, $P < 0.0001$). These results demonstrate that obtaining blood by fingerstick does not result in a clinically significant release of tissue factor. In addition, we did not observe any interference of plasma tissue factor with the whole blood prothrombin time assay. A direct relationship between tissue factor and age was observed. Am. J. Hematol. 55:193–198, 1997.

Key words: plasma tissue factor antigen; prothrombin time; capillaries

INTRODUCTION

Tissue factor is a cell membrane-associated single chain glycoprotein that serves as the receptor for factor VII [1,2]. The resulting complex between tissue factor and factor VII/VIIa catalyzes the conversion of factors X and IX to their active serine protease derivatives, leading to thrombin formation and ultimately to fibrin deposition. Thus, tissue factor is the main cellular initiator of the coagulation protease cascade. Under normal physiologic conditions, tissue factor activity is present at sites anatomically separated from flowing blood. In arteries, tissue factor activity is found predominantly in the tunica adventitia, which functions as an ''envelope'' sequestering tissue factor from the circulation [3–5]. This is in contrast to intravascular cells exposed to plasma, including normal, intact endothelium and peripheral blood cells, which express very little, if any, tissue factor ac-

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tivity unless they sustain physical or chemical damage [1–3,6]. Cellular mediators and agents including interleukin 1, endotoxins, tumor necrosis factor, and thrombin [7–12] have been shown to stimulate tissue factor activity in endothelial cells and monocytes. It has also been demonstrated that cultured cells such as fibroblasts, which, when intact, have little or no tissue factor activity, release tissue factor when disrupted or subjected to proteolysis [13].

Despite the reported sequestration of tissue factor to sites separated from circulating blood, several investigators have detected tissue factor in the plasma of healthy, normal volunteers [14–16]. Elevated levels of tissue factor have been found in several disease states including disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, vasculitis, renal failure treated with hemodialysis, and diabetic microangiopathy [14–16]. These findings suggest that plasma tissue factor activity may reflect endothelial injury or damage to other tissue factor containing cells.

It has been a long-held tenet that whole blood obtained by a fingerstick method (capillary blood) is unsuitable for coagulation time testing because it is presumed to contain high levels of tissue factor [17,18]. With the recent advances in portable capillary whole blood methods of measuring prothrombin time, patients on anticoagulation with warfarin can now monitor prothrombin time away from standard laboratory centers [19–21]. However, if the fingerstick process releases tissue factor, capillary measurements could result in a shortened prothrombin time leading to inadequate anticoagulation of patients. In order to determine the amount of tissue factor released during the process of specimen collection and its potential effect of shortening prothrombin time, plasma tissue factor antigen levels and prothrombin times were analyzed on paired samples of capillary and venous whole blood. In addition, capillary whole blood prothrombin time and its corresponding International Normalized Ratio (INR) measured by portable monitor were correlated to the plasma prothrombin time and INR determined by the standard one-stage clot based assay.

MATERIALS AND METHODS

Subjects

Thirty-three paired samples including 23 samples from 17 patients on chronic anticoagulation with warfarin being followed at the Thrombosis Center of UMDNJ-Robert Wood Johnson Medical School and 10 samples from 10 healthy controls were studied. Samples from patients who volunteered more than once were drawn on separate clinic visits and thus considered as independent observations. The most common indication for anticoagulation was deep venous thromboses (n = 10); other indications included prosthetic heart valves (n = 4), arterial thrombosis (n = 2), and atrial fibrillation (n = 1). The 10 controls were non-anticoagulated subjects without any acute or chronic illnesses, and were not taking any medications. The subjects gave written informed consent, and the study protocol was approved by the Institutional Review Board.

Blood Samples

Paired capillary (fingerstick) and venous blood samples were obtained from the subjects. Venous blood samples were collected by clean venipuncture into tubes containing 3.8% buffered sodium citrate (9 parts blood:1 volume anticoagulant) using a two-syringe technique. After the first 10–12 ml of blood was discarded, a second syringe was used to draw the blood samples for the study. Capillary whole blood samples were obtained by fingerstick technique using a lancing device (Tenderlett®, International Technidyne Corporation [ITC], Edison, NJ). After the first drop of blood was wiped off, approximately 100 μl of blood was obtained within 90 sec of incision.

Laboratory Methods

Venous blood samples for measurement of prothrombin time with a standard laboratory assay were centrifuged within 45 min of collection at 2,000*g* for 20 min at 4°C using a plasma separator to obtain platelet poor plasma. A one-stage clot-based assay using rabbit brain thromboplastin (ISI = 1.46) was performed using the ACL300 Plus laser-nephelometric centrifugal analyzer (Instrumentation Laboratories, Lexington, MA) and the INR was calculated [22].

Whole blood prothrombin times were determined by a battery-powered portable Hemochron® Jr. Microcoagulation System (ITC). Within 30 sec of obtaining the sample, a drop of blood (approximately 25 μl) was directly transferred to a 37°C-incubated cartridge. The instrument automatically sampled 15 μl of whole blood by a precision pump and coagulation was initiated once the blood specimen rehydrated the preloaded lyophilized rabbit brain thromboplastin (ISI 1.13). Clotting time was detected by a visual clot detection system and was mathematically converted to a plasma equivalent prothrombin time and INR.

For the measurement of tissue factor, blood samples obtained by venipuncture and fingerstick techniques were immediately aliquoted into microtubes containing 3.8% trisodium citrate (9 volumes of blood:1 volume of anticoagulant). Samples were centrifuged at 13,000 rpm for 5 min at room temperature in an Ependorf 5415 Centrifuge and the plasma was stored at −70°C. Tissue factor antigen levels were measured using an enzyme-linked immunoabsorbent assay (ELISA) (American Diagnostsica Inc., Greenwich, CT). This assay uses a murine anti-human tissue factor monoclonal antibody detecting tissue

TABLE I. Characteristics of Study Population

	Patients	Controls
Number	17	10
Age: Range (years)	19–88	28–42
Mean ± SD	52 ± 18	34 ± 5
Median	51	34
Gender		
Males, n(%)	10 (59%)	6 (60%)
Females, n(%)	7 (41%)	4 (40%)
Indications for warfarin therapy:		
DVT/PE, n(%)	10 (58%)	
Prosthetic valve, n(%)	4 (24%)	
Arterial thrombosis, n(%)	2 (12%)	
Atrial fibrillation, n(%)	1 (6%)	

factor apoprotein, tissue factor, and tissue factor-FVII complexes [23]. Briefly, plasma samples were diluted 1:10 in sample buffer consisting of 1% w/v bovine serum albumin. The diluted plasma samples were then incubated for 3 hr at room temperature in microtiter wells precoated with capture antibody. Tissue factor was detected using a biotinylated anti-human tissue factor antibody that specifically recognizes bound tissue factor, followed by streptavidin conjugated horseradish peroxidase and TMB substrate. The reaction was stopped by addition of sulfuric acid and absorbance was read at 450 nm. The lower limit of detection was 5 pg/ml of tissue factor antigen. Tissue factor was measured in plasma samples from three patients with disseminated intravascular coagulation as a positive control.

Statistical Methods

Statistical analysis was performed using Statistical Applications System Software (SAS Institute, Cary, NC). The effects of age, gender, and warfarin treatment on tissue factor levels were analyzed using analysis of covariance. Capillary and venous tissue factor levels and whole blood prothrombin times were compared with a paired *t*-test; INR, determined by fingerstick and venous whole blood samples, as well as venous plasma samples were compared using analysis of variance (ANOVA). Since each sample was an independent observation, more than one observation per patient was included for the *t*-test and the ANOVA. Results are reported as statistically significant if $P \leqslant 0.05$.

RESULTS

Study subjects on chronic warfarin therapy ranged from 19 to 88 years old, with a mean age of 52 years. There were ten males and seven females. Control subjects, six males and four females, ranged from 28 to 42 years of age with a mean age of 34 years (Table I). Indications for anticoagulation with warfarin included deep venous thrombosis with or without pulmonary embolism (n = 10, 58%), prosthetic heart valves (n = 4, 24%), arterial thrombosis (n = 2, 12%), and atrial fibrillation (n = 1, 6%). None of the patients had renal failure, disseminated intravascular coagulation, diabetic microangiopathy, or active vasculitis, conditions that are associated with increased tissue factor levels. One patient had a history of breast carcinoma in complete remission for 2 years after being treated with adjuvant chemotherapy and is currently on tamoxifen. One other patient had a history of resected non-invasive bladder carcinoma.

Anticoagulated study patients had higher venous tissue factor (235 ± 101 pg/ml) and capillary tissue factor (268 ± 106 pg/ml) than the controls (161 ± 42 pg/ml and 187 ± 63 pg/ml respectively, $P < 0.05$) (Table II). The difference between the two subject groups disappeared, however, after adjusting for age. A linear relationship between tissue factor levels and age was observed in the study population ($r = 0.76$, $P < 0.0001$, Fig. 1). This correlation persisted even after excluding the two patients with a history of malignancy. There were no effect on gender on tissue factor levels. Tissue factor levels determined from plasma samples of three subjects with disseminated intravascular coagulation (510, 659, and 1,430 pg/ml) were greater than the maximum levels observed in the study population.

Capillary tissue factor levels for the total study population were higher than the venous tissue factor levels (243 ± 102 pg/ml vs. 213 ± 93 pg/ml, $P = 0.0001$), with a mean difference of 31 pg/ml. Higher tissue factor levels in capillary samples were also observed when anticoagulated and control subjects were analyzed separately (Table II). In addition, whole blood prothrombin times measured with the portable monitor demonstrated a small but statistically significant decrease in capillary samples compared to the venous samples (17.7 ± 5 sec vs. 18.3 ± 5.4 sec, $P = 0.004$). Similarly, whole blood INR was lower for the capillary samples compared to the venous samples (2.33 ± 1.37 vs. 2.52 ± 1.56, $P < 0.05$). Importantly, however, there was no correlation between the differences in capillary and venous tissue factor levels and the differences in capillary and venous whole blood prothrombin time. Capillary tissue factor and INR also were not correlated (Fig. 2). The same observations held true after separate analysis of anticoagulated patients and controls. Prothrombin time and INR determined by portable monitor in both capillary and venous blood were highly correlated with the prothrombin time and INR determined by using the laboratory clot-based assay ($r = 0.98$, $P < 0.0001$).

DISCUSSION

In 1935 when Quick and colleagues [18] described the one-stage prothrombin time, it was emphasized that un-

TABLE II. Tissue Factor and Prothrombin Time Measurements in Capillary and Venous Samples From Anticoagulated and Control Subjects

	All n = 33	Anticoagulated n = 23	Control n = 10
Capillary Samples			
Tissue factor (pg/ml)	243 ± 102*	268 ± 106****	187 ± 63****
Prothrombin time (sec)	17.7 ± 5.0**	20.0 ± 4.4	12.6 ± 0.3
INR	2.33 ± 1.37***	2.88 ± 1.3	1.07 ± 0.07
Venous Samples			
Tissue factor (pg/ml)	213 ± 93*	235 ± 101*****	161 ± 42*****
Prothrombin time (sec)	18.3 ± 5.4**	20.7 ± 4.8	12.8 ± 0.06
INR	2.52 ± 1.56***	3.12 ± 1.51	1.13 ± 0.10

Values are mean ± SD. Comparisons were made using Student's t-test.
*p = 0.0001, **p = 0.004, ***p = 0.003, ****p<0.025, *****p<0.05

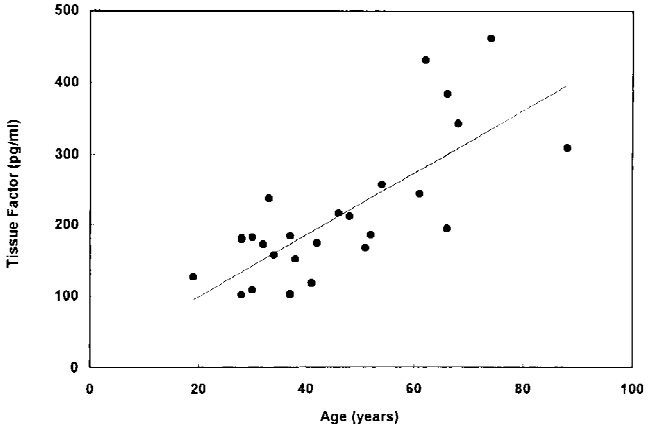

Fig. 1. Relationship between tissue factor and age (r = 0.76, *P* < 0.0001). Tissue factor was determined as described in Materials and Methods.

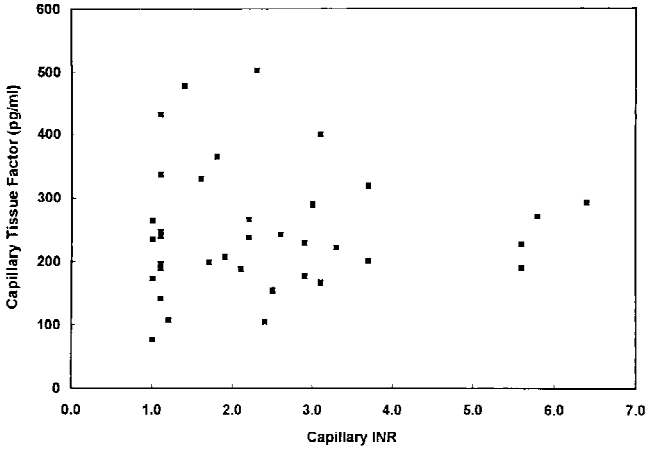

Fig. 2. Relationship between capillary tissue factor and capillary INR.

necessary trauma during venipuncture should be avoided and that capillary whole blood is not suitable for coagulation time testing. It was hypothesized that contamination of capillary whole blood samples with tissue thromboplastin during skin puncture could shorten the clotting time, making such samples unreliable for coagulation testing [17,18]. In support of this view, Lee and White [24] demonstrated a shorter coagulation time of capillary blood (5 min) compared to venous blood (6.5 min) in twenty-four healthy subjects. This premise has been upheld by several other investigators [25–27], establishing it as a dominant view regarding coagulation time measurements. This long-held tenet has been challenged by the recent development of portable monitors to determine prothrombin time using capillary whole blood obtained by fingerstick.

In a study published by Drake et al. [4], tissue factor was shown to be localized predominantly in the tunica adventitia and was undetectable in the endothelium. Capillary and post-capillary venules did not exhibit any tissue factor activity, whereas epidermis expressed tissue factor intensely. Some form of cellular trauma, either physical or chemical such as endotoxin or inflammatory reaction, is required for the full expression of tissue factor by the endothelium. Thus, the presence of tissue factor intravascularly was considered to be a pathologic state [2]. In the current study, however, we were able to demonstrate tissue factor in the plasma of normal controls consistent with the findings of other investigators [14–16]. Tissue factor levels in normal healthy volunteers (161 ± 42 pg/ml, mean ± SD) were within the range observed by Takahashi et al. [14] (138 ± 51 pg/ml), Wada et al. [15] (126 ± 41 pg/ml), and Koyama et al. [16] (149 ± 72 pg/ml). Since the first 10 ml of venous blood was discarded, cellular trauma during the process of venipuncture would not have solely accounted for the detection of tissue factor in the venous specimens. Although the origin of plasma tissue factor remains unclear, it has been theorized, at least in part, to results from its detachment from cellular membranes [16] or from its constant generation by activated monocytes and macrophages [28].

We were able to observe higher tissue factor levels in capillary whole blood obtained by fingerstick compared to venipuncture-derived samples. This probably results

from more cellular trauma during the fingerstick and milking process. This small but statistically significant difference, however, did not correlate with the duration of specimen collection (data not shown) or with the differences in prothrombin time and INR of the capillary whole blood compared to the venous blood. Increases in factor VIIa correlating with increases in thrombomodulin, a measure of endothelial injury but not with increases in tissue factor, have been observed in uremic subjects [29] and may provide an alternate explanation for the decreases in capillary PT we have seen. The observed differences between capillary and venous prothrombin time and INR, although statistically significant, were not of clinical importance. The high correlation of prothrombin time and INR between our laboratory using venous blood and the portable monitor utilizing capillary whole blood confirms the findings of other studies which also have demonstrated high correlation between portable monitors and laboratory based testing [19,30].

In the present study a linear correlation between age and tissue factor levels was seen, which is in contrast to the results reported by Koyama et al. [16]. The data are consistent with the hypothesis that age-related atherosclerotic vessel changes may result in a higher basal tissue factor generation [12,31]. The clinical significance of these findings remains to be elucidated.

The results demonstrate that plasma contains a detectable level of tissue factor antigen. Fingerstick sampling with milking resulted in a statistically significant but small release of tissue factor which was not shown to interfere with the whole blood prothrombin time. In addition, capillary whole blood prothrombin time was highly correlated with the standard plasma-based assay. These data support the concept that whole blood fingerstick sampling is clinically reliable for prothrombin time measurements.

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